nature portfolio

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|---|---|
| ☐ | ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Photos of phenotypes were collected through Nikon camera.<br>Fluorescence signals were detected by using a laser confocal microscopy (LSM 880).<br>Images of western blotting were collected with K 4000 (Beijing kcrx bio-company).<br>RNAseq was performed on Illumina high-throughput sequencing platform NovaSeq 6000 (Novogene, Beijing, China).<br>luciferase activity was detected using a low-light cooled CCD imaging apparatus (Lumina II, USA).<br>Real-time PCR was performed using SYBR Green real-time PCR master mix (TaKaRa) on a CFX96 real-time PCR detection system (Bio-Rad). |
| Data analysis | Structural model for SnRK2.2/2.3 created by AlphaFold2 (www.alphafold.ebi.ac.uk).<br>Proteins band intensity was determined by ImageJ.<br>Statistical analyses were performed with Microsoft Excel.<br>Fluorescence image was analyzed with ZEN 2.3 (Zeiss). |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Sequence data for the genes described in this article can be found in the Arabidopsis Information Resource (https://www.arabidopsis.org).
Structural model for SnRK2.2/2.3 created by AlphaFold2 (www.alphafold.ebi.ac.uk).
The RNA-seq data of previous experiments under accession GSE74864 (GSM1936759, GSM1936760, GSM1936761, GSM1936762, GSM1936763, GSM1936764, GSM1936765, GSM1936766, GSM1936767).

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | Not applicable |
| Reporting on race, ethnicity, or other socially relevant groupings | Not applicable |
| Population characteristics | Not applicable |
| Recruitment | Not applicable |
| Ethics oversight | Not applicable |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | Sample size was determined based on general standards for biological studies and requirements for statistical analysis, attempting to have a minimum of n = 3 biological replicates with sufficient reproducibility. |
| Data exclusions | No data were excluded. |
| Replication | All experiments were repeated two-three biologically independent experiments as mentioned in figure legends and methods. |
| Randomization | Samples were allocated randomly for imaging and analyses. There was no bias in selecting particular cells within an image. |
| Blinding | Not applicable |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☐ | ☒ Plants |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# Antibodies

Antibodies used

Anti-His: TransGen Biotech, HT501-01
Anti-GFP: Abcam, ab290
Anti-SnRK2.2/2.3/2.6:Agrisera, AS14 2783
Anti-H4: Abcam, EPR16599
Anti-GST: TransGen Biotech, HT601-01
Anti-FLAG: proteintech, 20543-1-AP
Anti-actin:TransGen Biotech, HC201-01
Anti-Phosphoserine/threonine antibody: EMC biosciences, PP2551
HRP conjugated anti-rabbit IgG: TransGen Biotech, HS101-01
HRP conjugated anti-mouse IgG: TransGen Biotech, HS201-01
HRP conjugated ConA:Sigma-Aldrich, L6397

Validation

Anti-His: https://www.transgen.com/antibody_tag/385.html
Anti-GFP: https://www.abcam.cn/products/primary-antibodies/gfp-antibody-ab290.html
Anti-SnRK2.2/2.3/2.6: https://www.agrisera.com/en/artiklar/srk-ser-thr-protein-kinase-snrk.html
Anti-H4: https://www.abcam.cn/products/primary-antibodies/histone-h4-antibody-epr16599-chip-grade-ab177840.html
Anti-GST: https://www.transgen.com/antibody_tag/388.html
Anti-FLAG: https://www.ptgcn.com/products/Flag-Tag-Antibody-20543-1-AP.htm
Anti-actin: https://www.transgen.com/antibody_reference/393.html
Anti-Phosphoserine/threonine antibody: https://ecmbio.com/collections/phospho-specific-antibodies/products/pp2551
HRP conjugated anti-rabbit IgG: https://www.transgen.com/antibody_second/397.html
HRP conjugated anti-mouse IgG: https://www.transgen.com/antibody_second/403.html
HRP conjugated ConA:https://www.sigmaaldrich.cn/CN/zh/product/sigma/l6397

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | 293T cells (Cleveland Lab stock, ATCC) |
| Authentication | 293T cells for producing recombinant proteins G?β-3×FLAG |
| Mycoplasma contamination | Not applicable |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study. |

# Plants

Seed stocks

Arabidopsis thaliana plants used in this study were in the Columbia-0 (Col-0) background.Transfer DNA insertion lines, g?β-2 (SALK_120812), g?β-3 (SALK_093735), g?β-4 (SALK_039458), stt3a-2 (CS800052), rsw3 (SALK_124837C), fut11 (SALK_087481), cgl1-T (SALK_073650) and hgl1-2 (SALK_141821) were obtained from the Arabidopsis Biological Resource Center (ABRC),and stt3a-2/snrk2.2/2.3, rsw3/snrk2.2/2.3, fut11/snrk2.2/2.3, cgl1-T/snrk2.2/2.3, hgl1-2/snrk2.2/2.3 was obtained by crossing stt3a-2, rsw3, fut11, cgl1-T, hgl1-2 with snrk2.2/2.3. In addition, g?β-2/snrk2.2/2.3/2.6 were screened the F2 for quadruple homozygous progeny, after g?β-2 and snrk2.2/2.3/2.6 were crossed.

Novel plant genotypes

Complementation lines (pro:1-1.com/2) were generated by transforming g?β-2 with pG?β::G?β-GFP by Agrobacterium-mediated transformation. Overexpression lines under the control of the 35S promoter were generated by transforming Col-0

Authentication

All transgenic lines were selected by antibiotic resistance and the expression levels of the genes were verified.